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Metabolomic analysis of serum alpha-tocopherol among men in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study

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Abstract

Background/Objectives: The role of vitamin E in chronic disease risk remains incompletely understood, particularly in an un-supplemented state, and evidence is sparse regarding the biological actions and pathways involved in its influence on health outcomes. Identifying vitamin-E-associated metabolites through agnostic metabolomics analyses can contribute to elucidating the specific associations and disease etiology. This study aims to investigate the association between circulating metabolites and serum α-tocopherol concentration in an un-supplemented state.

Subjects/Methods: Metabolomic analysis of 4,294 male participants was conducted based on pre-supplementation fasting serum in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study. The associations between 1,791 known metabolites measured by ultra-high-performance LC-MS/GC-MS and HPLC-determined α-tocopherol concentration were estimated using multivariable linear regression. Differences in metabolite levels per unit difference in α-tocopherol concentration were calculated as standardized β-coefficients and standard errors.

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Author Contributions

Conception and design: WRL, JNS, DA. Development of methodology: WRL, JH, JNS, DA. Acquisition of data WRL, SJW, DA. Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): WL, JL, JH, SJW, JNS, DA. Writing, review, and/or revision of the manuscript: WRL, JL, JH, JNS, SJW, DA.

Competing Interests

The authors declare no competing interests.

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Code availability: The analytical methods for this study are available from the corresponding author upon appropriate request.

Results: A total of 252 metabolites were associated with serum α-tocopherol at the Bonferroni-corrected *p*-value ($p<2.79\times10^{-5}$). Most of these metabolites were of lipid and amino acid origin, with the respective subclasses of dicarboxylic fatty acids, and valine, leucine, and isoleucine metabolism, being highly represented. Among lipids, the strongest signals were observed for linoleoyl-arachidonoyl-glycerol (18:2/20:4)[2](β=0.149; $p=8.65\times10^{-146}$) and sphingomyelin (D18:2/18:1) (β=0.035; $p=1.36\times10^{-30}$). For amino acids, the strongest signals were aminoadipic acid (β=0.021; $p=5.01\times10^{-13}$) and *L*-leucine (β=0.007; $p=1.05\times10^{-12}$).

Conclusions: The large number of metabolites, particularly lipid and amino acid compounds associated with serum α-tocopherol provide leads regarding potential mechanisms through which vitamin E influences human health, including its role in cardiovascular disease and cancer.

Keywords

Metabolomics; Vitamin E; Alpha-Tocopherol; Metabolism; Sphingolipid Metabolism; Sphingomyelin; Lipid

Introduction

The importance of vitamin E to disease risk and mortality has been investigated through observational studies, controlled trials, and laboratory studies.[1] The related literature suggests that antioxidants such as vitamin E and other tocopherols/tocotrienols offer protection against tissue oxidative injury, thereby contributing to the prevention of various chronic diseases.[1] As an essential micronutrient, vitamin E is primarily available through diet and supplement use.[2,3] In humans, α-tocopherol is the predominant form of vitamin E in tissues and circulation. Although findings from experimental studies suggest that α-tocopherol may inhibit the development of cancer and its progression, the mechanism(s) are not well understood.[4]

Epidemiologic studies that have examined the associations between vitamin E status and chronic disease risk are inconsistent for cardiovascular disease and cancer. For instance, a recent large prospective analysis observed that increased α-tocopherol concentrations were associated with lower lung cancer risk, whereas some controlled trials have reported that α-tocopherol supplementation did not reduce lung cancer incidence.[5–7] Similarly, studies have reported that vitamin E intake or α-tocopherol concentrations were inversely associated with cardiovascular-related risk and mortality,[8–10] however, a large meta-analysis of clinical trials showed no impact of vitamin E supplementation on incident cardiovascular disease or deaths.[11] Such findings highlight the inconsistencies in the relationship between vitamin E status and human health, and point to the need for a deeper and more comprehensive understanding of the effects of vitamin E on biological pathways relevant to human health and disease outcomes. Identifying the potential mechanisms of action for α-tocopherol would provide greater insight into its role in the development of chronic diseases and may inform prevention research, especially among groups that are at greater risk for cardiovascular disease and cancer. By identifying low-molecular weight serum metabolites associated with α-tocopherol concentrations in an un-supplemented state, the present study aims to contribute to the knowledge of how vitamin E compounds might inhibit or modulate the pathogenesis of such chronic diseases.

Materials and methods

Study Design

The Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study was a double-blind, placebo-controlled primary cancer prevention trial that investigated whether β-carotene or α-tocopherol supplementation lowered cancer incidence. The ATBC Study design has been described in detail elsewhere.[12] Briefly, this 2×2 factorial randomized controlled trial included 29,133 male Finnish smokers, ages 50–69, who smoked at least five cigarettes daily. Each participant received either β-carotene (20 mg/day), α-tocopherol (50 mg/day), both β-carotene and α-tocopherol, or placebo for 5–8 years (median of 6.1) until the study concluded on April 30, 1993. Lifestyle and medical history data, including smoking history and alcohol consumption, were obtained through detailed questionnaires at study baseline, along with a validated food-frequency questionnaire that was administered. Height and weight were measured. Pre-randomization (i.e., pre-supplementation) blood samples were also obtained during baseline visits from all participants (after an overnight fast), processed to serum, protected from light, and stored at −70°C until assayed. All participants provided written informed consent. The ATBC Study was approved by the institutional review boards at the Finnish National Public Health Institute and U.S. National Cancer Institute and conducted in accordance with human subject guidelines and regulations.

The present cross-sectional analysis is based on participants included in several case-control and other sub-studies nested within the ATBC Study (subsequently referred to as "metabolomic sets").[13–20] After removing duplicate participants, the final analysis included 4,294 men (Supplemental Figure 1).

Laboratory measurement of serum α-tocopherol and metabolites

Baseline serum α-tocopherol concentrations were measured by isocratic high-performance liquid chromatography (LC) from 1986–1988 in the biochemistry laboratory of the National Public Health Institute in Helsinki, Finland. Using a non-targeted approach, serum metabolites were assayed at Metabolon, Inc. (Durham, N.C.) using ultrahigh performance LC/mass spectrometry (MS) and gas chromatography (GC)/MS as previously described in detail, including sample preparation, quality control, data extraction, and compound identification.[13–15,21] Briefly, each 150μl sample was analyzed using GC-MS and LC-MS/MS (+electrospray ionization [ESI]) and LC-MS/MS(-ESI). Using an aqueous methanol extraction procedure, all samples were processed to 1) extract proteins, 2) separate small molecules bound to protein or trapped in the precipitated protein matrix, and 3) recover chemically diverse metabolites. To ensure extraction efficiency, methanol contained four recovery standards (4-chlorophenylalanine, D6-cholesterol, DL-2-fluorophenylglycine, and tridecanoic acid). For positive ion analysis, two aliquots of each sample were reconstituted utilizing a 50μl 0.1% formic acid in water (pH approximately 3.5). For negative ion analysis, the other two aliquots were reconstituted in 50μ of 6.5mM ammonium bicarbonate in water (pH 8). Subsequent extracts were separated into fractions for analysis by GC/MS, ultrahigh performance LC/MS/MS (negative mode), and ultra-performance and LC/MS/MS (positive mode). To remove organic solvent, samples were briefly transferred to TurboVap (Zymark). Under a vacuum, samples were separately frozen and dried. Samples were then

arranged for the appropriate instrument (ultrahigh performance LC/MS/MS or GC/MS). Using Metabolon's instruments (software and hardware), raw data were extracted, peak identified, and quality control (QC) processed. Internal controls consisted of an extraction process (five recovery standards), injection (up to eleven standards), and to control for experimental variability, an alignment standard for quality assurance was used. We were able to identify compounds through comparison with library entries of purified standards or recurrent unknown entities. Additionally, biochemical identifications are based on accurate mass match to the library ± 0.005 amu, retention time/index (RI) within a slight margin RI window of the proposed identification, and MS/MS forward and reverse scores between authentic standards and experimental data. The scores from the MS/MS were derived from comparison between ions present in the experimental spectrum and in the library spectrum. Over 2,400 commercially available identifiable standard compounds were acquired and used for the LC and GC platform for determining analytical characteristics.[22] To standardize batch variability, signal strength was divided by the batch median value for each metabolite and subject, after which they were log-transformed and normalized. Metabolite values that were below the limit of detection within each metabolomic set were imputed to have the minimum of all non-missing values.

Following exclusion of metabolites with fewer than ten non-missing values across all metabolomics sets or single value metabolites in an individual metabolomic set, 1,791 identified compounds were included in this analysis. Based on standard chemical classes, each of the 1,226 metabolites were categorized as one of the following ten mutually exclusive classes: amino acids and amino acid derivatives (herein referred to as "amino acids"), carbohydrates, cofactors and vitamins, energy, lipid, nucleotide, peptides, partially characterized molecules, secondary metabolism, and xenobiotics. Included within each batch were blinded quality control duplicate pooled samples to evaluate the technical reliability of data, and coefficients of variation (CV) were calculated. Across metabolites, the median and interquartile range (IQR) for CV% was 9%, in line with those previously observed for blood samples analyzed by the same laboratory, and documented a high reliability among the metabolomics platform used in this study.[21–23]

Statistical analysis

We report continuous variables as the mean ± standard deviation (SD) and categorical variables as relative frequencies. We performed multivariable linear regression to examine the association between independently assayed serum α-tocopherol concentration and each metabolite adjusting for age at randomization, body mass index (BMI), serum total cholesterol, metabolomic set, case-control status, and smoking intensity (number of cigarettes/day). We estimated standardized beta-coefficients as the change in SD units in metabolite signal strength per 1 SD increase in serum α-tocopherol concentration. For each model, standardized beta-coefficients and corresponding standard errors were computed. We applied Bonferroni correction using $p=0.05/1,791=2.79 \times 10^{-5}$ as the adjusted significance threshold.

We also conducted biochemical pathway Gene Set Analysis (GSA) to evaluate whether pre-defined metabolic super-pathways and sub-pathways were related to serum α-tocopherol

within the metabolomic sets.[24] With $\{z_1,\ldots,z_s\}$ as the Z values test from S metabolites in predefined chemical pathways, GSA evaluates the "maxmean" statistic max $(-z^+, -z^-)$ that is the average of all Z values positive (negative) values and calculates the p values by 10,000 permutations. Additionally, Gaussian graphical models (GGMs) were created to summarize relationships among metabolites in the pathways associated with serum α-tocopherol. Direct relationships between metabolites by pathways were measured with a partial correlation coefficient, where each correlation is conditioned on other metabolites, less than −0.2 or greater than 0.2 from the analysis.

All analyses were performed using SAS 9.4, except for GSA and plots, which were conducted using R 4.0.2. Finally, all statistical tests and reported p-values were two-sided.

Results

Baseline characteristics of the 4,294 participants included in this analysis are shown in Table 1. The median serum concentrations of α-tocopherol and total cholesterol were 11.9 mg/L (±3.2) and 6.2 mmol/L (±1.1), respectively. Mean smoking duration for the study population was 37.9 years and 28% of the men smoked 20 cigarettes daily. Most men on average were overweight (mean, SD BMI 26.0±3.7) and among those who exercised, 20% did so at least three times per week.

In the multivariable linear regression, 668 serum metabolites were associated with α-tocopherol concentration at the nominal $p<0.05$ level of which 252 remained associated after Bonferroni correction ($p<2.79 \times 10^{-5}$) (Supplemental Figure 2, Supplemental Table 1, Table 2 [non-lipid metabolites], and Table 3 [lipid metabolites]). Most amino acids were positively associated with a 1-SD increase in α-tocopherol with the strongest signal for aminoadipic acid (β=0.021 per SD increase in α-tocopherol concentration; $p=5.01 \times 10^{-13}$) (Table 2). Seven amino acid metabolites were inversely associated with α-tocopherol concentration: 2-ethylhydracrylic acid, β=−0.015; pyroglutamine, β=−0.019; 2-hydroxy-3-methylbutyric acid, β=−0.032; tiglylglycine, β= −0.048; ureidopropionic acid, β=−0.014; pipecolic acid, β=−0.038; and acetylglycine, β=−0.031). For cofactors and vitamin metabolites, the strongest association was observed for γ-tocopherol/β-tocopherol (β=0.068 per SD increase in α-tocopherol concentration; $p=7.31\times10^{-80}$). Among xenobiotics, nearly all metabolites were positively associated with α-tocopherol, with the strongest association for hydroxypropanedioic acid (β=0.051 per SD increase in α-tocopherol concentration; $p=4.99 \times 10^{-20}$). Interestingly, we observed that 3D,7D,11D-phytanic acid in the food component/plant chemical sub-class was inversely associated with α-tocopherol (β=−0.037 per SD increase in α-tocopherol concentration; $p=7.75 \times 10^{-10}$) (Table 2).

Table 3 presents multivariable linear regression findings for lipids associated with serum α-tocopherol concentration after Bonferroni correction for multiple comparisons. The strongest positive association was with linoleoyl-arachidonoyl-glycerol (18:2/20:4) [2] (β=0.149 per SD increase in α-tocopherol concentration; $p=8.65 \times 10^{-146}$). The most frequently associated (both positively and inversely) chemical sub-class was sphingolipid metabolism. We observed that plasmogens and dicarboxylic fatty acids were largely

inversely associated with α-tocopherol, including dodecanedioic acid (β=−0.044 per unit SD increase in α-tocopherol concentration; $p=2.78 \times 10^{-6}$).

Unknown metabolites (i.e., "X_" compounds) significantly associated with α-tocopherol concentration after Bonferroni correction are presented in Supplemental Table 1 along with all other metabolites. To examine whether serum α-tocopherol or the metabolite-serum α-tocopherol associations was influenced by the diagnosis of cancer after the baseline blood collection, we conducted a sensitivity analysis that was restricted to the non-cases within the metabolomics sets (n=1,410) and observed that the top associated metabolites remained largely unchanged (Supplemental Table 2), though there were fewer overall associated metabolites.

In the GSA, we observed that the chemical super-pathways of cofactors/vitamins and lipids were associated with serum α-tocopherol at the $p<0.05$ threshold ($p=0.003$ and $p=0.027$, respectively; Table 4). Examination by chemical sub-pathway, dicarboxylic fatty acids, bile acid metabolites, and diacyglycerols were associated with serum α-tocopherol at the $p<0.05$ threshold ($p=2.00 \times 10^{-4}$, $p=0.011$, and $p=0.014$, respectively). However, no metabolite chemical super- and sub-pathway exceeded the Bonferroni threshold of $p=4.26 \times 10^{-5}$. Among the chemical super-pathways that were associated at $p<0.05$, we developed GGMs for metabolites with conditional correlations ($r \leq -0.2$ or ≥ 0.2) (Figure 1). For lipids, we stratified by chemical sub-pathways that were associated with serum α-tocopherol.

Discussion

The present agnostic analysis of nearly 4,300 Finnish men identified 252 metabolites in several chemical classes that were associated with serum α-tocopherol concentration after stringent correction for multiple comparisons. To the best of our knowledge, this is the first investigation of metabolomic profiling of serum α-tocopherol in the un-supplemented state. We observed that lipids and amino acids were the most highly represented metabolites, with linoleoyl-arachidonoyl-glycerol (18:2/20:4) [2] and aminoadipic acid ranking first within the two chemical classes, respectively. In addition, arabonate/xylonate, γ-tocopherol/β-tocopherol, and hydroxypropanedioic acid were the top metabolites within the carbohydrate, cofactors/vitamins, and xenobiotic chemical classes, respectively.

Aminoadipic acid is involved in glucose homeostasis and considered a biomarker for risk of diabetes; it has also been associated with prostate cancer risk in one recent study.[25,26] Also associated with serum α-tocopherol was the amino acid derivative 3-methylhistidine which is associated with BMI, red meat consumption, and prostate cancer risk, being a reported biomarker of muscle turnover and meat intake based on the important role of the histidine metabolism pathway in protein synthesis.[18,27,28] Among the few metabolites inversely related to serum α-tocopherol, pyroglutamine was previously reported to be associated with reduced prostate cancer risk, pipecolate may indicate healthy dietary habits including consumption of fruits and nuts, and tiglylglycine has a documented role in renal function as a potential indicator of glomerular disease.[26,29–31]

Among cofactors and vitamins, the chemical sub-class γ-tocopherol was associated with serum α-tocopherol, aligning with previous studies.[32] Both α-tocopherol and γ-tocopherol are key among the eight fat-soluble vitamin E compounds (i.e., tocopherols [α-, β-, γ-, δ-] and tocotrienols [α-, β-, γ-, δ-]).[33] Although γ-tocopherol is the most common vitamin E compound in Western diets based on high vegetable oil consumption, α-tocopherol is the predominant, preferentially bio-active moiety in human metabolism and the primary form in vitamin E supplements.[1,33]

Our findings regarding xenobiotic metabolites showed a strong α-tocopherol association with piperine. Research suggests that this biochemical may have anticancer properties.[34] Further, both piperine and α-tocopherol have reported anti-inflammatory effects.[35] Interestingly, 3D,7D,11D-phytanic acid, a potential fatty acid metabolic intermediate and a food component/plant metabolite, was inversely associated with serum α-tocopherol and has been studied in relation to prostate cancer risk, although the precise associations with cancer remain inconclusive.[36,37]

Not unexpectedly based on vitamin E blood transport in lipoproteins, lipids were most represented in association with vitamin E status in our study. Functionally, α-tocopherol is able to donate phenolic hydrogens to inhibit lipid radical production and peroxidation.[1,38] The diacylglycerol (DAG) linoleoyl-arachidonoyl-glycerol (18:2/20:4) [2] had the strongest association with circulating α-tocopherol. The potential biological significance of this metabolite is not well understood, although the DAG pathway was suggested to influence risk of metabolic disorders and have a key role in lipid-induced insulin resistance.[39,40] Diacylglycerols function as lipid intermediates in metabolism and as membrane-associated bioactive second messengers, the latter potentially related to our observation that sphingolipids were highly represented among the α-tocopherol-associated lipids.[41] Sphingolipid metabolism has a significant role in regulating inflammatory signaling pathways, including that dietary sphingolipids are suggested to have anti-inflammatory effects, potentially inhibiting inflammation-related chronic diseases.[42,43] We also observed several ceramide metabolite associations. Ceramides have key roles in sphingolipid metabolism and are associated with elevated risk of type 2 diabetes, atherosclerosis, and other metabolic disorders.[44] Additionally, stearoyl-linoleoyl-glycerophosphocholine (1), palmitoyl-linoleoyl-glycerophosphoinositol (1), and docosadienoate (22:2N6) metabolites were associated with α-tocopherol and documented in a prospective metabolomic analysis to reduce prostate cancer risk.[13] We also observed stearoyl-linoleoyl-glycerophosphoethanolamine was associated with α-tocopherol, and reported to decrease risk of aggressive prostate cancer (defined as TNM stage III-IV, AJCC stage ≥3 or Gleason sum ≥8).[13] Similarly, in a prospective metabolomic study, methyl palmitate (15 or 2) metabolite was associated with α-tocopherol and shown to reduce the risk of colorectal cancer in men, potentially by complimenting α-tocopherol in buffering against oxidative stress and inflammatory response.[45] Finally, our findings also showed that α-tocopherol was associated with lysoPC (18:2(9Z,12Z)). LysoPCs have been documented in prior studies to influence apoptosis among proliferating cancer cells, where a recent study observed that lysoPC (18:2) was associated with reduced incident liver cancer in men.[46]

In humans, α-tocopherol is considered the most biologically active form of vitamin E, and after intake primarily from animal product foods, it is preferentially metabolized from the portal circulation and stored in the liver for ongoing tissue requirements.[1] By contrast, other vitamin E compounds, especially γ-tocopherol, are derived from dietary sources such as vegetable oils, seeds, fruit, whole grains, and nuts.[2,3] Additionally, vitamin E supplementation can elevate serum α-tocopherol concentration, though findings on increasing via supplementation and cancer risk remain inconsistent.[6,7,47,48] However, prior studies reported that increasing serum α-tocopherol through dietary modification instead of supplementation was associated with reduced lung cancer risk.[5,48] Further, findings on the impact α-tocopherol has on cardiovascular event and cardiovascular-related mortality remains heterogonous, where outcome potentially varies by vitamin E supplementation dosage.[1,10,11,49,50]

Although our study provides new insight into the association between physiological serum α-tocopherol and low molecular weight circulating metabolites, several limitations should be noted. First, the study population was comprised of male smokers of majority European ancestry, potentially limiting generalizability of findings to other populations including non-smokers, women, and other racial and ethnic groups. Second, this study is cross-sectional in nature with blood collection occurring at one (baseline) time-point and was not interventional, thereby limiting our ability to infer causal inferences between serum α-tocopherol and metabolites; i.e., higher serum α-tocopherol may have impacted specific metabolites and pathways, or some metabolites influenced α-tocopherol metabolism. Third, residual confounding related to health and underlying diseases is possible, although we extensively adjusted for additional potential confounders in our final models, including high-density lipoprotein (HDL) cholesterol, and alcohol consumption at baseline after which the metabolite associations remained unchanged compared with our final model. Fourth, it is possible that the magnitude of the association between α-tocopherol and metabolites were influenced by length of serum storage. However, small metabolites are more stable during longer storage duration at ultra-low temperatures, such as the present study. Finally, our results should be interpreted with caution as we present statistical associations and not direct biological relationships; therefore, further laboratory and clinical biological validation of our findings is needed to confirm the associated pathways between α-tocopherol and the circulating metabolites identified.

Our study limitations are offset by notable strengths, including a large sample size for a metabolomic analysis of this kind. The baseline serum used for analysis was obtained after an overnight fast, and both the α-tocopherol HPLC assay and agnostic metabolomic platform were state-of-the-science technologies and had been validated. We identified nearly 2,000 metabolites across the many biochemical pathways, some of which have been associated with cancer risk and cardiometabolic health.

Conclusions

Using an agnostic approach, the present study identified a large number of metabolites in various metabolic pathways associated with serum α-tocopherol concentrations, some of which have been suggested to be related to risk of cardiovascular disease, metabolic

disorders, and cancer (particularly prostate cancer). The most highly represented chemical classes were lipids, amino acids, and xenobiotics (e.g., vitamin-related compounds), providing potential insights into the biological mechanisms relevant to the role of vitamin E status in human health and chronic disease pathogenesis that require biological validation. Re-examination of the identified metabolite associations in other more diverse populations is warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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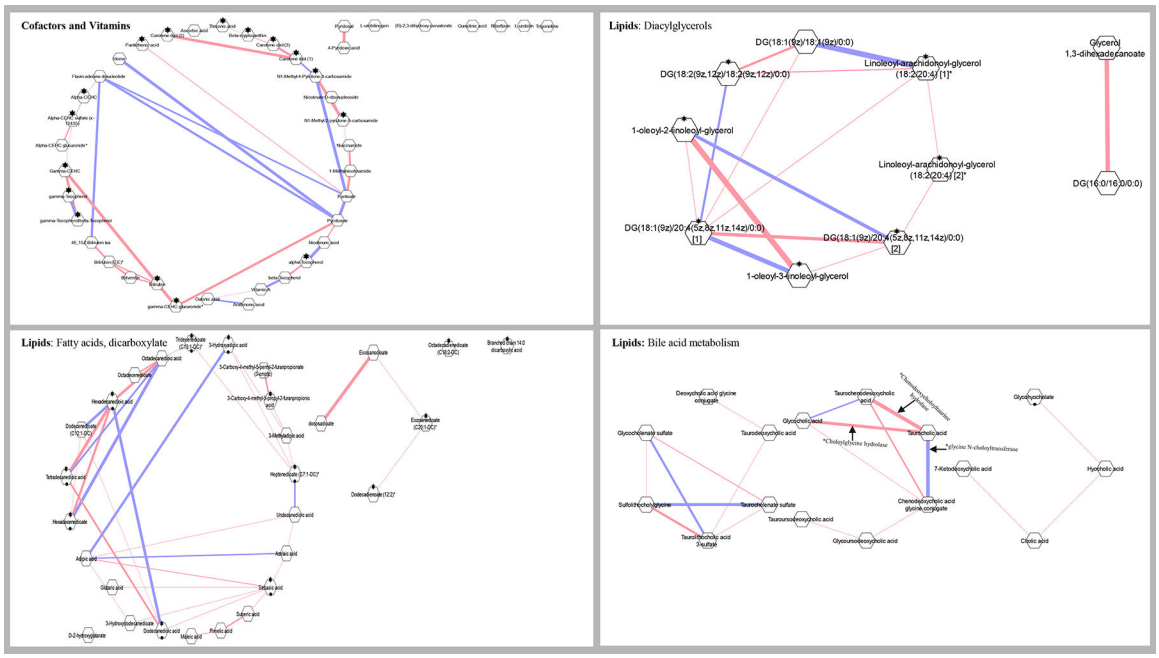

Figure 1.

Gaussian graphical models of metabolites among associated chemical super-pathways and sub-pathways most related to serum alpha-tocopherol concentration in the study. Metabolites are drawn as hexagons, and the pairs with an absolute value of conditional correlation ≥0.2 are connected by a line. The colors represent the association directions of conditional correlations, with pink indicating positive conditional correlations, and blue indicating negative conditional correlations. Magnitudes of the conditional correlations are represented by line width (i.e., wider lines for stronger correlations). Metabolites with an "●" indicates an inverse association with serum α-tocopherol and "★" indicates that the association met the Bonferroni correction threshold $p<2.79 \times 10^{-5}$. Enzyme on the pathway between metabolites are indicated by "*".

Table 1.

Selected baseline characteristics of 4,294 Finnish men in the ATBC Study

Age at randomization (years), mean (SD)	57.8 (5.1)
Height (cm), mean (SD)	173.7 (6.2)
Weight (kg), mean (SD)	79.3 (12.6)
BMI (kg/m^2), mean (SD)	26.0 (3.7)
Cigarettes smoked/day, mean (SD)	20 (8.5)
Duration of smoking (years), mean (SD)	37.9 (8.6)
Physical exercise in leisure time (3 or more times/week, %)	849 (19.8)
Dietary intake/day, mean (SD)	
Total energy (kcal)	2700 (741)
Total fat (g)	123.9 (40.4)
Calcium (mg)	1398 (552)
Fruit (g)	93.5 (84.3)
Coffee (g)	620 (349.6)
Serum biochemistry, mean (SD)	
α-tocopherol (mg/L)	11.9 (3.2)
Retinol (μg/L)	592 (126)
Total cholesterol (mmol/L)	6.2 (1.1)
HDL cholesterol (mmol/L)	1.2 (0.3)

Table 2.

Non-lipid metabolites associated with serum α-tocopherol concentration at 2.79×10^{-5} level of statistical significance (adjusted for age at randomization, BMI, cigarettes per day, serum total cholesterol, metabolomics sets, and case-control status).

Chemical Class and Metabolite	Chemical Sub-Class	Estimate	Standard error	p value
Amino Acids				
Aminoadipic acid	Lysine metabolism	0.021	0.0029	5.01×10^{-13}
L-Leucine	Valine, Leucine, and Isoleucine metabolism	0.007	0.0009	1.05×10^{-12}
Creatine	Creatine metabolism	0.021	0.0033	2.34×10^{-10}
2-Ethylhydracrylic acid	Valine, Leucine, and Isoleucine Metabolism	−0.015	0.0023	2.95×10^{-10}
Acetylglycine	Glycine, Serine and Threonine Metabolism	−0.031	0.0050	7.88×10^{-10}
L-Lysine	Lysine metabolism	0.007	0.0012	1.17×10^{-09}
Pyroglutamine	Glutamate metabolism	−0.019	0.0032	2.37×10^{-09}
N-Acetylleucine	Valine, Leucine, and Isoleucine metabolism	0.013	0.0022	3.76×10^{-09}
2-Oxoarginine	Urea Cycle; Arginine and Proline metabolism	0.020	0.0035	6.01×10^{-09}
2-Aminobenzoic acid	Tryptophan metabolism	0.019	0.0034	3.97×10^{-08}
Argininic acid	Urea Cycle; Arginine and Proline Metabolism	0.019	0.0034	6.88×10^{-08}
Isovalerylcarnitine	Valine, Leucine, and Isoleucine metabolism	0.012	0.0023	1.00×10^{-07}
Isobutyryl-L-carnitine	Valine, Leucine, and Isoleucine metabolism	0.019	0.0037	1.98×10^{-07}
5'-Methylthioadenosine	Polyamine metabolism	0.013	0.0026	2.35×10^{-07}
1-Carboxyethylleucine	Leucine, Isoleucine, and Valine metabolism	0.024	0.0050	1.62×10^{-06}
2-Hydroxy-3-methylbutyric acid	Valine, Leucine, and Isoleucine metabolism	−0.032	0.0070	3.62×10^{-06}
Kynurenic acid	Tryptophan metabolism	0.018	0.0040	6.08×10^{-06}
Urea	Urea Cycle; Arginine-, Proline-, metabolism	0.009	0.0020	6.76×10^{-06}
Tiglylglycine	Leucine, Isoleucine, and Valine metabolism	−0.048	0.0106	6.90×10^{-06}
1-Carboxyethylisoleucine	Leucine, Isoleucine, and Valine metabolism	0.023	0.0052	8.02×10^{-06}
3-Methylhistidine	Histidine metabolism	0.084	0.0192	1.25×10^{-05}
N-Acetyl-3-methylhistidine	Histidine metabolism	0.051	0.0119	2.04×10^{-05}
Ureidopropionic acid	Alanine, and Aspartate metabolism	−0.014	0.0032	2.54×10^{-05}
L-Cysteine	Cysteine, Methionine, Sam, Taurine metabolism	0.009	0.0020	2.77×10^{-05}
Pipecolic acid	Lysine metabolism	−0.038	0.0091	2.78×10^{-05}

Chemical Class and Metabolite	Chemical Sub-Class	Estimate	Standard error	p value
Carbohydrates				
Arabonate/xylonate	Pentose metabolism	0.021	0.0034	8.84×10^{-10}
Oxalic acid	Glyoxylate And Dicarboxylate metabolism	0.065	0.0107	1.39×10^{-09}
Glyceric acid	Glycolysis, Gluconeogenesis, Pyruvate metabolism	0.026	0.0044	2.69×10^{-09}
Cofactors and Vitamins				
γ-Tocopherol/β-tocopherol	Tocopherol metabolism	0.068	0.0036	7.31×10^{-80}
α-tocopherol	Tocopherol metabolism	0.059	0.0037	1.20×10^{-57}
Carotene diol (1)	Vitamin A metabolism	0.036	0.0033	4.25×10^{-28}
Gamma-CEHC	Tocopherol metabolism	0.120	0.0114	5.99×10^{-26}
γ-Tocopherol	Tocopherol metabolism	0.084	0.0098	1.42×10^{-17}
Gamma-CEHC glucuronide	Tocopherol metabolism	0.080	0.0097	2.22×10^{-16}
Beta-Cryptoxanthin	Vitamin A metabolism	0.102	0.0126	6.66×10^{-16}
Carotene diol (2)	Vitamin A metabolism	0.027	0.0038	6.25×10^{-13}
Pantothenic acid	Pantothenate and COA metabolism	0.032	0.0047	6.44×10^{-12}
Carotene diol (3)	Vitamin A metabolism	0.040	0.0058	7.53×10^{-12}
Threonic acid	Ascorbate and, Aldarate metabolism	0.074	0.0109	1.44×10^{-11}
Alpha-CEHC sulfate (X_12435)	Tocopherol metabolism	0.090	0.0183	7.93×10^{-07}
N1-Methyl-2-pyridone-5-carboxamide	Nicotinate and Nicotinamide metabolism	0.046	0.0096	2.01×10^{-06}
4E,15Z-Bilirubin IXA	Hemoglobin and Porphyrin metabolism	0.013	0.0031	1.14×10^{-05}
N1-Methyl-4-pyridone-3-carboxamide	Nicotinate and Nicotinamide metabolism	0.047	0.0107	1.20×10^{-05}
Energy				
Isocitric acid	TCA Cycle	0.017	0.0035	1.82×10^{-06}
Nucleotides				
Uric acid	Purine metabolism, Urate metabolism	0.010	0.0016	1.98×10^{-09}
9-Methyluric acid	Purine metabolism, Urate metabolism	0.040	0.0070	8.23×10^{-09}
Partially Characterized Molecules				
Glycineconjugateofc10H14O2(1)	Partially characterized molecules	0.108	0.0092	4.06×10^{-32}
Xenobiotics				
Hydroxypropanedioic acid	Bacterial/Fungal	0.051	0.0056	4.99×10^{-20}
Ergothioneine	Food Component/Plant	0.045	0.0053	9.49×10^{-18}
Hydroxy-CMPF	Chemical	0.032	0.0042	3.71×10^{-14}
Piperine	Food Component/Plant	0.149	0.0224	3.13×10^{-11}
Glucuronide of piperine metabolite C17H21NO3 (4)	Food Component/Plant	0.074	0.0112	4.99×10^{-11}

Chemical Class and Metabolite	Chemical Sub-Class	Estimate	Standard error	p value
Sulfate of piperine metabolite C16H19NO3 (3)	Food Component/Plant	0.050	0.0077	6.33×10^{-11}
Perfluorooctanesulfonic acid	Chemical	0.027	0.0041	6.95×10^{-11}
Sulfate of piperine metabolite C16H19NO3 (2)	Food Component/Plant	0.062	0.0096	7.79×10^{-11}
Glucuronide of piperine metabolite C17H21NO3 (3)	Food Component/Plant	0.054	0.0084	1.36×10^{-10}
3D,7D,11D-Phytanic acid	Food Component/Plant	−0.037	0.0059	7.75×10^{-10}
Glucuronide of piperine metabolite C17H21NO3 (5)	Food Component/Plant	0.055	0.0089	1.01×10^{-09}
Perfluorooctanoic acid	Chemical	0.032	0.0061	1.84×10^{-07}
Sulfate of piperine metabolite C18H21No3 (1)	Food Component/Plant	0.036	0.0070	3.69×10^{-07}
3-Hydroxystachydrine	Food Component/Plant	0.267	0.0621	1.74×10^{-05}

Table 3.

Lipid metabolites associated with serum α-tocopherol concentration at 2.79×10^{-5} level of statistical significance (adjusted for age at randomization, BMI, cigarettes per day, serum total cholesterol, metabolomics sets, and case-control status).

Metabolite	Chemical Sub-Class	Estimate	Standard error	*p* value
Linoleoyl-arachidonoyl-glycerol (18:2/20:4) [2]	Diacylglycerol	0.149	0.0058	8.65×10^{-146}
Linoleyl carnitine	Fatty acid metabolism (Acyl carnitine)	0.074	0.0036	5.32×10^{-93}
DG (18:1(9Z)/20:4(5Z,8Z,11Z,14Z)/0:0) [1]	Diacylglycerol (DAG)	0.119	0.0059	5.11×10^{-89}
PC (18:0/18:2(9Z,12Z))	Phosphatidylcholine (PC)	0.021	0.0011	7.63×10^{-81}
1-(1-Enyl-palmitoyl)-2-oleoyl-GPC (P-16:0/18:1)	Plasmalogen	−0.035	0.0019	3.19×10^{-77}
DG(18:2(9Z,12Z)/18:2(9Z,12Z)/0:0)	Diacylglycerol	0.316	0.0177	6.26×10^{-71}
1-Linoleoylglycerophosphoinositol Or 1-Linoleoyl-GPI (18:2)	Lysolipid	0.055	0.0033	3.67×10^{-62}
PC (16:0/18:2(9Z,12Z))	Phosphatidylcholine (PC)	0.014	0.0008	3.55×10^{-61}
PI (18:0/18:2(9Z,12Z))	Phosphatidylinositol (PI)	0.056	0.0035	3.52×10^{-59}
PC (P-16:0/16:1(9Z))	Plasmalogen	−0.037	0.0023	1.71×10^{-58}
1-Oleoyl-3-linoleoyl-glycerol	Diacylglycerol	0.172	0.0116	3.45×10^{-50}
Linoleoyl-arachidonoyl-glycerol (18:2/20:4) [1]	Diacylglycerol	0.192	0.0130	4.71×10^{-49}
3-Decenoylcarnitine	Fatty acid metabolism (Acyl carnitine, monounsaturated)	0.060	0.0042	6.87×10^{-45}
Palmitoyl-linoleoyl-glycerol (16:0/18:2) [2]	Diacylglycerol	0.174	0.0135	2.53×10^{-38}
PI (16:0/18:2(9Z,12Z))	Phosphatidylinositol (PI)	0.046	0.0036	2.35×10^{-37}
MG (0:0/18:1(9Z)/0:0)	Monoacylglycerol (MAG)	0.086	0.0067	3.75×10^{-37}
Sphingomyelin (D18:2/18:1)	Sphingolipid metabolism	0.035	0.0031	1.36×10^{-30}
PI (18:0/20:4(5Z,8Z,11Z,14Z))	Phosphatidylinositol (PI)	0.025	0.0022	1.23×10^{-29}
PC (18:1(9Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	Phosphatidylcholine (PC)	0.023	0.0021	5.22×10^{-29}
PE (18:0/20:4(5Z,8Z,11Z,14Z))	Phosphatidylethanolamine (PE)	0.053	0.0048	1.29×10^{-28}
Alpha or gamma linolenic acid	Essential Fatty acid	0.043	0.0042	3.74×10^{-25}
MG (22:6(4Z,7Z,10Z,13Z,16Z,19Z)/0:0/0:0)	Monoacylglycerol	0.067	0.0065	4.31×10^{-25}
1-Oleoyl-2-linoleoyl-glycerol	Diacylglycerol	0.166	0.0164	5.67×10^{-24}
4-Methylhexanoylglutamine	Fatty acid metabolism (Acyl glutamine)	−0.074	0.0074	2.06×10^{-23}
Linoleic acid	Essential Fatty acid	0.045	0.0046	1.37×10^{-22}
Eicosenedioate (C20:1-DC)	Fatty acid, Dicarboxylate	0.098	0.0103	1.19×10^{-21}
Sphingomyelin (D18:2/23:1)	Sphingolipid metabolism	−0.020	0.0020	1.57×10^{-21}
Behenoylcarnitine (C22)	Fatty acid metabolism (Acyl carnitine)	0.033	0.0035	2.59×10^{-21}
Lignoceroylcarnitine (C24)	Fatty acid metabolism (Acyl Carnitine)	0.026	0.0028	1.59×10^{-20}

Metabolite	Chemical Sub-Class	Estimate	Standard error	*p* value
PE (16:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	Phosphatidylethanolamine (PE)	0.072	0.0078	2.94×10^{-20}
Cis-4-decenoyl carnitine or Cis-4-decenoylcarnitine (C10:1)	Carnitine metabolism	0.089	0.0099	1.35×10^{-19}
Sphingomyelin (D18:1/17:0, D17:1/18:0, D19:1/16:0)	Sphingolipid metabolism	−0.017	0.0019	2.48×10^{-19}
7Z,10Z-Hexadecadienoic acid	Long chain polyunsaturated fatty acid (N3 and N6)	0.034	0.0038	3.89×10^{-19}
PE (16:0/20:4(5Z,8Z,11Z,14Z))	Phosphatidylethanolamine (PE)	0.036	0.0041	7.83×10^{-19}
Gamma-linolenyl carnitine	Fatty acid metabolism (Acyl carnitine)	0.046	0.0052	9.05×10^{-19}
PC (P-16:0/16:0)	Plasmalogen	−0.014	0.0016	1.73×10^{-18}
3-Carboxy-4-methyl-5-propyl-2-furanpropionic acid (CMPF)	Fatty acid, Dicarboxylate	0.041	0.0048	1.03×10^{-17}
Campesterol	Sterol/Steroid	0.053	0.0063	3.21×10^{-17}
Dihomo-linoleoylcarnitine (C20:2)	Fatty acid metabolism (Acyl carnitine)	0.031	0.0037	3.35×10^{-17}
1-Palmitoyl-2-dihomo-linolenoyl-GPC (16:0/20:3)	Phosphatidylcholine (PC)	0.010	0.0012	4.01×10^{-17}
1-Dihomo-linolenylglycerol (20:3) or 1-Dihomo-linolenylglycerol (Alpha, Gamma)	Monoacylglycerol	0.051	0.0063	4.44×10^{-16}
Ximenoylcarnitine (C26:1)	Fatty acid metabolism (Acyl Carnitine)	0.025	0.0031	1.11×10^{-15}
Sphingomyelin (D17:1/16:0, D18:1/15:0, D16:1/17:0) or sphingomyelin (D18:1/15:0, D16:1/17:0)	Sphingolipid metabolism	−0.019	0.0024	2.00×10^{-15}
N-Stearoyl-sphingadienine (D18:2/18:0) or X – 24204	Ceramides	0.027	0.0034	6.88×10^{-15}
PE (18:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	Phosphatidylethanolamine (PE)	0.087	0.0115	2.93×10^{-14}
1-Arachidonoylglycerophosphoinositol	Lysolipid	0.019	0.0026	3.60×10^{-14}
Tridecenedioate (C13:1-DC)	Fatty acid, Dicarboxylate	−0.029	0.0038	5.06×10^{-14}
N-Palmitoyl-sphingadienine (D18:2/16:0)	Sphingolipid metabolism	0.017	0.0023	7.86×10^{-14}
N-Palmitoylserine	Endocannabinoid	−0.023	0.0031	2.10×10^{-13}
1-Linoleoyl-GPG (18:2)	Lysophospholipid	0.062	0.0085	2.25×10^{-13}
Glycosyl ceramide (D18:1/23:1, D17:1/24:1)	Hexosylceramides (HCER)	−0.037	0.0052	4.25×10^{-13}
N-Behenoyl-sphingadienine (D18:2/22:0)	Sphingolipid metabolism	0.041	0.0059	1.83×10^{-12}
Glycosyl-N-tricosanoyl-sphingadienine (D18:2/23:0)	Ceramides	−0.025	0.0036	1.99×10^{-12}
PE (P-18:0/18:2(9Z,12Z))	Plasmalogen	0.019	0.0028	4.18×10^{-12}
Palmitoyl-sphingosine-phosphoethanolamine (D18:1/16:0)	Ceramide PES	−0.014	0.0020	6.74×10^{-12}
SM (D18:1/22:0)	Sphingolipid metabolism	0.010	0.0015	1.76×10^{-11}
Palmitoyl sphingomyelin	Sphingolipid	−0.006	0.0009	1.99×10^{-11}
N-Acetylaminooctanoic Acid	Fatty acid, Amino	0.024	0.0036	2.04×10^{-11}
Sphingomyelin (D18:2/23:0, D18:1/23:1, D17:1/24:1)	Sphingolipid metabolism	−0.012	0.0018	2.38×10^{-11}
Xi-17-methyloctadecanoic scid	Fatty acid, Branched	−0.035	0.0053	4.33×10^{-11}

Metabolite	Chemical Sub-Class	Estimate	Standard error	*p* value
SM (D18:0/14:0)	Sphingolipid metabolism	−0.027	0.0043	4.52×10^{-10}
PC (16:0/18:0)	Phosphatidylcholine (PC)	0.014	0.0023	7.89×10^{-10}
Nervonoylcarnitine (C24:1)	Fatty acid metabolism (Acyl carnitine)	0.023	0.0037	1.02×10^{-09}
8,11,14-Eicosatrienoic acid	Essential fatty acid	0.016	0.0026	1.30×10^{-09}
Glycosyl-N-stearoyl-sphingosine or glycosyl-N-stearoyl-sphingosine (D18:1/18:0)	Ceramides	−0.015	0.0025	1.60×10^{-09}
Hexanoylglutamine or X_12824	Fatty acid metabolism (Acyl glutamine)	−0.040	0.0067	2.34×10^{-09}
N-Oleoylserine	Endocannabinoid	−0.017	0.0029	2.97×10^{-09}
Hexadecenedioate	Fatty acid, Dicarboxylate	−0.045	0.0077	3.83×10^{-09}
Sphingomyelin (D18:2/21:0, D16:2/23:0)	Sphingolipid metabolism	−0.016	0.0027	4.07×10^{-09}
Lysope (0:0/18:2(9Z,12Z))	Lysolipid	0.031	0.0052	4.15×10^{-09}
Undecylenic acid	Medium chain fatty acid	−0.016	0.0028	6.14×10^{-09}
PE (18:0/18:2(9Z,12Z))	Phosphatidylethanolamine (PE)	0.085	0.0147	8.62×10^{-09}
Dodecadienoate (12:2)	Fatty acid, dicarboxylate	0.073	0.0128	1.15×10^{-08}
1-Linoleoylglycerol (1-Monolinolein) or 1-Linoleoylglycerol (18:2)	Monoacylglycerol	0.096	0.0169	1.33×10^{-08}
Myo-inositol 1-phosphate	Inositol metabolism	0.023	0.0041	1.64×10^{-08}
Ceramide (D18:2/24:1, D18:1/24:2)	Ceramides	0.025	0.0045	2.29×10^{-08}
1-Stearoylglycerophosphoinositol	Lysolipid	0.016	0.0030	2.63×10^{-08}
Cholesterol	Sterol/Steroid	0.004	0.0008	2.88×10^{-08}
Tetradecanedioic acid	Fatty acid, Dicarboxylate	−0.054	0.0097	3.41×10^{-08}
Palmitoyl dihydrosphingomyelin (D18:0/16:0)	Sphingolipid metabolism	−0.009	0.0017	3.47×10^{-08}
Docosahexaenoylcholine	Fatty acid metabolism (Acyl choline)	0.028	0.0050	3.73×10^{-08}
Sphingomyelin (D18:1/19:0, D19:1/18:0)	Sphingolipid metabolism	−0.013	0.0024	3.96×10^{-08}
Sphingomyelin (D18:1/20:2, D18:2/20:1, D16:1/22:2)	Sphingolipid metabolism	0.017	0.0032	4.37×10^{-08}
MG (18:1(9Z)/0:0/0:0)	Monoacylglycerol	0.099	0.0182	4.95×10^{-08}
PC (18:2/18:2)	Phosphatidylcholine (PC)	0.034	0.0063	5.02×10^{-08}
SM (D18:1/18:0)	Sphingolipid	−0.008	0.0015	5.67×10^{-08}
Myristoleic acid	Long chain fatty acid	−0.048	0.0089	6.90×10^{-08}
Eicosadienoic acid	Long chain fatty acid	0.019	0.0035	6.95×10^{-08}
Heptenedioate (C7:1-DC)	Fatty acid, Dicarboxylate	−0.021	0.0038	7.51×10^{-08}
Cis-4-decenoate	Medium chain fatty acid	0.064	0.0120	9.97×10^{-08}
SM (D18:1/14:0)	Sphingolipid metabolism	−0.014	0.0026	1.02×10^{-07}
Dihomo-linolenoylcarnitine (20:3N3 Or 6)	Fatty acid metabolism (Acyl carnitine)	0.020	0.0038	1.08×10^{-07}
LysoPE (16:0/0:0)	Lysolipid	0.019	0.0035	1.43×10^{-07}
5-Dodecenoic acid	Medium chain fatty acid	−0.041	0.0078	1.44×10^{-07}

Metabolite	Chemical Sub-Class	Estimate	Standard error	*p* value
Octadecadienedioate (C18:2-DC)	Fatty acid, Dicarboxylate	0.036	0.0068	1.83×10^{-07}
LysoPE (18:2(9Z,12Z)/0:0)	Lysolipid	0.028	0.0055	2.53×10^{-07}
PC (16:0/22:6)	Phosphatidylcholine (PC)	0.009	0.0017	3.30×10^{-07}
9,10-Dhome	Fatty acid, Dihydroxy	0.034	0.0066	3.42×10^{-07}
3-Hydroxysebacic acid	Fatty acid, monohydroxy	−0.057	0.0113	3.89×10^{-07}
Linoleoyl ethanolamide	Endocannabinoid	0.074	0.0146	4.00×10^{-07}
Hexadecanedioic acid	Fatty acid, Dicarboxylate	−0.046	0.0091	4.69×10^{-07}
Sphingomyelin (D17:1/14:0, D16:1/15:0)	Sphingolipid metabolism	−0.027	0.0053	5.14×10^{-07}
Stearoyl-linoleoyl-glycerophosphoethanolamine (1)	Lysolipid	0.076	0.0153	6.90×10^{-07}
Stearoyl-linoleoyl-glycerophosphocholine (1)	Lysolipid	0.021	0.0042	6.95×10^{-07}
DG (18:1(9Z)/20:4(5Z,8Z,11Z,14Z)/0:0) [2]	Diacylglycerol	0.094	0.0190	7.22×10^{-07}
Dl-2-Aminooctanoic acid	Fatty acid, Amino	0.016	0.0033	7.76×10^{-07}
PE (P-16:0/18:1(9Z))	Plasmalogen	−0.012	0.0024	8.77×10^{-07}
Methyl palmitate (15 or 2) or 15-Methylpalmitate (Isobar with 2-methylpalmitate)	Fatty acid, Branched	−0.027	0.0056	9.66×10^{-07}
Branched Chain 14:0 Dicarboxylic Acid	Fatty acid, Dicarboxylate	0.026	0.0053	1.05×10^{-06}
Lathosterol	Sterol/Steroid	0.022	0.0045	1.22×10^{-06}
LysoPC (18:2(9Z,12Z))	Lysolipid	0.016	0.0033	1.37×10^{-06}
PE (16:0/18:2(9Z,12Z))	Phosphatidylethanolamine (PE)	0.074	0.0153	1.39×10^{-06}
1-Stearoyl-GPG (18:0) or 1-Stearoylglycerophosphoglycerol	Lysolipid	0.023	0.0048	1.46×10^{-06}
7Alpha-hydroxy-3-oxo-4-cholestenoate	Sterol/Steroid	0.011	0.0024	1.57×10^{-06}
Glyco-beta-muricholate	Primary bile acid metabolism	−0.109	0.0227	1.62×10^{-06}
Propionylcarnitine	Fatty acid metabolism (Also BCAA metabolism)	0.011	0.0022	1.76×10^{-06}
Lysope (0:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	Lysolipid	0.019	0.0039	1.97×10^{-06}
Glycosyl-N-palmitoyl-sphingosine or Glycosyl-N-palmitoyl-sphingosine (D18:1/16:0)	Ceramides	−0.009	0.0019	2.26×10^{-06}
Lactosyl-N-nervonoyl-sphingosine (D18:1/24:1)	Ceramides	−0.011	0.0024	2.28×10^{-06}
Dodecanedioic acid	Fatty acid, Dicarboxylate	−0.044	0.0094	2.78×10^{-06}
Sebacic acid	Fatty acid, Dicarboxylate	−0.058	0.0124	2.79×10^{-06}
Linoleoylcholine or X_11537	Fatty acid metabolism (Acyl choline)	0.050	0.0107	2.90×10^{-06}
Capric acid	Medium chain fatty acid	−0.041	0.0088	3.32×10^{-06}
(R)-2-Hydroxycaprylic acid	Fatty acid, Monohydroxy	0.012	0.0027	3.85×10^{-06}
3-Hydroxyadipic acid	Fatty acid, Dicarboxylate	−0.033	0.0073	4.51×10^{-06}
SM (D18:0/22:0)	Sphingolipid metabolism	0.017	0.0036	5.31×10^{-06}
MG (0:0/18:2(9Z,12Z)/0:0)	Monoacylglycerol	0.064	0.0141	5.37×10^{-06}
Beta-sitosterol	Sterol/Steroid	0.037	0.0082	5.44×10^{-06}
MG (0:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z)/0:0)	Monoacylglycerol	0.052	0.0116	6.60×10^{-06}

Metabolite	Chemical Sub-Class	Estimate	Standard error	*p* value
Sphingomyelin (D18:1/25:0, D19:0/24:1, D20:1/23:0, D19:1/24:0)	Sphingolipid metabolism	−0.011	0.0026	9.44×10^{-06}
3-Hydroxybutyroylglycine	Fatty acid metabolism (Acyl glycine)	−0.052	0.0118	1.05×10^{-05}
5Alpha-pregnan-3beta,20alpha-diol disulfate	Sterol/Steroid	−0.021	0.0048	1.24×10^{-05}
Docosahexaenoic acid	Essential fatty acid	0.016	0.0036	1.27×10^{-05}
Palmitoleoyl sphingomyelin or Sphingomyelin (D18:2/16:0, D18:1/16:1)	Sphingolipid metabolism	0.005	0.0012	1.27×10^{-05}
LysoPC(18:0)	Lysolipid	0.018	0.0041	1.58×10^{-05}
Eicosenoyl sphingomyelin or Sphingomyelin (D18:1/20:1, D18:2/20:0)	Sphingolipid metabolism	0.008	0.0019	1.59×10^{-05}
Choline	Glycerolipid metabolism	0.007	0.0016	1.89×10^{-05}
4-Trimethylammoniobutanoic acid	Carnitine metabolism	−0.006	0.0014	2.30×10^{-05}
2-Hydroxydecanoate or 2-hydroxydecanoic acid	Fatty acid, Monohydroxy	−0.024	0.0056	2.31×10^{-05}
Palmitoyl-linoleoyl-glycerophosphoinositol (1)	Lysolipid	0.042	0.0099	2.43×10^{-05}
Docosadienoate (22:2N6)	Long chain fatty acid	0.015	0.0037	2.44×10^{-05}
16-Hydroxy-hexadecanoic acid	Fatty acid, Monohydroxy	−0.018	0.0042	2.44×10^{-05}

Abbreviations: MAG, Monoacylglycerol; PC, Phosphatidylcholine; PE, Phosphatidylethanolamine; PI, Phosphatidylinositol; HCER, Hexosylceramides

Table 4.

Gene set analysis for chemical class and chemical sub-class of metabolites for association with serum α-tocopherol concentration ($P<0.05$)

	Number of contributing metabolites	*P* value
Super-Pathway		
Cofactors and vitamins	41	0.003
Lipids	529	0.027
Sub-Pathway		
Fatty acids, dicarboxylate	29	2.00×10^{-04}
Bile acid metabolism	16	0.011
Diacylglycerols	11	0.014

Note: Bonferroni threshold level of statistical significance $p = 4.26 \times 10^{-5}$